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11/30/94

Project No. _____

Book No. _____

TITLE _____

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Proposed: Amplify pGAPDH from strand - changing and
~~different~~ w different enzymes again.

GAPDH / Regular Forward + Reverse primers / Deep Vent worked
 - never tried with Tag + Deep Vent,
 - but no problem with Tag alone (pg 88).

used: Deep Vent buffer - 2 mM Mg
 200 μ M dNTP
 0.5 μ M primer
 100 pg template (10 pg/1 μ)

Tag:	Unit:	<u>Tag + Deep Vent</u>				<u>Deep Vent</u>	
		31	32	10 + .001	.005	15 .16	.17 .18
1	2	0	33	34			
3	4	.5	35	36	.01		
5	6	1	37	38	.05		
7	8	1.5	39	40	.1		
9	10	2	41	42	.2		
11	12	2.5	43	44	.5		
13	14	5	29	30	1		
15			17	X			
	15x					45 46	47 48

10X buffer	75	Cocktail T	→ 50 μ l/Rx	20 X
dNTP	15			1
Template	150		+ 50 μ l	
primers 1	3.75	enzyme	di. ammount	+ t
2	3.75	D.V. + 1U Tag in all	di. am	2
				dep. rev
50 μ l/Rx				

added enzyme
 separately in 1 μ l

T Pag

Witnessed & Understood by me,

Date /

12/14/94

Invented by

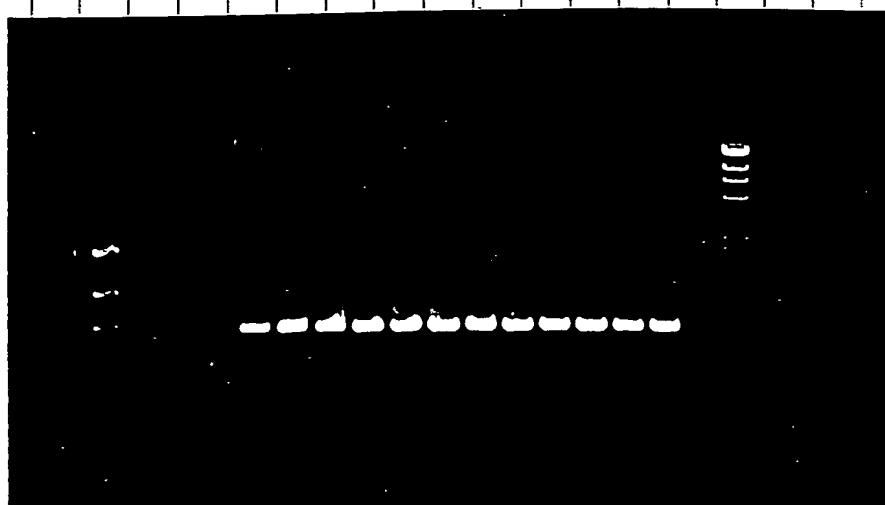
Recorded by

Date

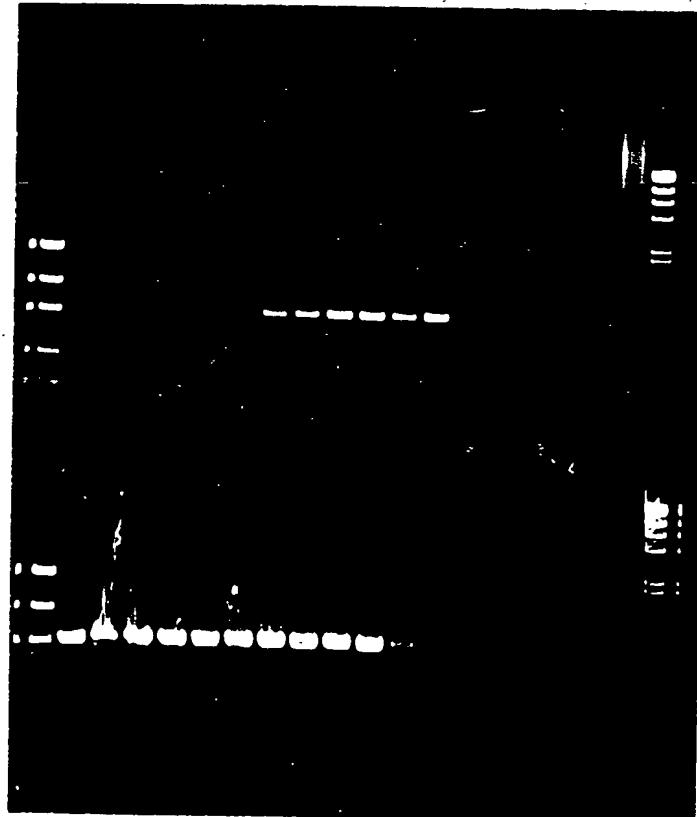
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ag N. _____

Tag



0 .5 1 1.5 2 2.5 5
 .001 .005 .01 .05 .1 .2 .5 1 2



1 U Tag .001 .005 .01 .05 .1 .2 .5 1 U Deyuent

Result:

- 100 pg + 2.5 cycles seem to be enough
- Deyuent alone with regular primers worked again
- Deyuent at lower concentration works better. With 0.05 U good product yield was seen.
- So may be worth each run, if the enzyme cost is lower, it might have worked with Deyuent. Try?

T Page No. _____

Signed & Understood by me,

Date

12/19/94

Invented by

Recorded by

V. Sharanan

Date

12/1/94